LLNL-TR-667015

Lawrence Livermore National Laboratory

Report for WIPP UG Sample #3, R15C5 (9/3/14)

Forensic Science Center

January 28, 2015

Disclaimer

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Section 1

Background

On December 10, 2014, LLNL received a 0.16-g sample of fine, solid material from WIPP. The sample was forwarded by SRNL and its identifier, as given by SRNL, was "WIPP UG Sample #3, R15C5 (9/3/14); SRNL LIMS# 300313812; LLNL ID: RC-12-04-14-01". After extraction, the LLNL sample identification given to this sample was FSC 14-14-1-A-1.

The sample was solvent extracted and the sample extract was analyzed using gas chromatography/mass spectrometry (GC/MS), gas chromatography coupled with nitrogen-specific detection (GC-N), gas chromatography coupled with flame photoionization detection (GC-FPD), and liquid chromatography/mass spectrometry (LC/MS). The goals of the analyses were to identify any organic compounds that might be present in the samples and to specifically look for evidence of nitrated organic compounds.

Section 2

Sample Preparation and Analysis

Using standard protocols, the entire 0.16-g sample (along with a method blank) was extracted, sequentially, with three, 5-mL aliquots of ultra-high purity methanol. The sample-solvent system was extracted by mixing for approximately 2 minutes with a vortex mixer and the resulting extract was centrifuged for approximately 5 minutes to separate the solids from the solvent. Centrifugation also minimized transfer of radioactivity into the solvent. The resulting sample extracts were combined and reduced to a final volume of 1 mL, using a gentle stream of clean N_2. The LLNL identifier for this sample extract was FSC 14-14-1-A-1. For GC/MS analyses, aliquots of this extract were injected directly. For LC/MS analyses, the methanol sample extract and method blank samples were diluted 1:50 with ultrapure water prior to analysis.

The sample extract was analyzed by gas chromatography/mass spectrometry (GC/MS), gas chromatography coupled with nitrogen-specific detection (GC-N), gas chromatography coupled with flame photoionization detection (GC-FPD), and liquid chromatography/mass spectrometry (LC/MS). The goals of the analyses were to identify any organic compounds that might be present in the samples and to specifically look for evidence of nitrated organic compounds.

The samples were first screened with GC-based techniques, all using the same separation conditions, as described below:

GC Instrument manufacturer and type: Agilent 6890 GC
Carrier gas: Helium
Flow rate: 1.30 mL/min
Injection mode: Splitless, 0.75 min
Injector temperature: 250 °C
Column: Agilent HP-5 MS UI: (5% diphenyl 95% dimethyl polysiloxane)
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature program: 40 °C (3 min), 8 °C/min, 300 °C (3 min)

Additionally, GC/MS conditions were as follows:

MS Instrument manufacturer and type: Agilent 5973
Solvent delay time: 3 minutes
Electron energy: 70 eV
Mass resolution: 0.6 u
Scan range: 29-600 *m/z* in 0.4 min
Source temperature: 250 °C

Both LC/MS and LC/MS/MS analyses were done, in separate experiments, using an Agilent 1290 Infinity LC coupled with an Agilent 6530 Q-TOF (high-resolution) mass spectrometer. LC separation conditions were as follows:

Injection volume: 5 µL
Eluent composition: A = H_2O with 0.1% formic acid
B = acetonitrile with 0.1% formic acid
Elution program: 95% A for 5 min, linear gradient to 20% A in 10 min, hold at 20% A for 10 min, regenerate column by returning to 95% A in 3 min, hold for 10 min
Flow rate: 200 µL/min
Column brand/phase: Waters Atlantis T3/C18
Column Length x ID x Particle size: 150 mm x 2.1 mm x 3 µm
Column temperature: 30 °C

Detection of analytes was performed using both positive and negative electrospray ionization modes (separate experiments). For positive electrospray, the following conditions were used:

Ionization type: Electrospray
Ionization polarity: Positive
Capillary Voltage: 3750 V
Nozzle Voltage: 500 V
Scan range/time: *m/z* 50-1000 in 333 ms (3 spectra/sec)

For negative electrospray, the following conditions were used:

Ionization type: Electrospray
Ionization polarity: Negative
Capillary Voltage: 5500 V
Nozzle Voltage: 1500 V
Scan range/time: *m/z* 50-1000 in 333 ms (3 spectra/sec)

For tandem mass spectrometry (MS/MS) experiments in both positive and negative electrospray ionization modes, the auto MS/MS product ion scan was used to fragment the three most abundant ions, with variable collision energies (set as a function of the precursor ion mass) and scan range/time of *m/z* 30-1000 in 1 sec.

Section 3

Results

The samples were first screened by GC-N and GC-FPD to determine if any semi-volatile organic compounds were present that contained the elements nitrogen, sulfur, or phosphorous. No nitrogen, phosphorous, or sulfur-containing compounds were detected in these analyses that could be specifically attributed to the sample. GC/MS analysis was also used as a screening tool, primarily because mass spectral databases and structure elucidation from first principles could be used for compound identification and to inform LC/MS analyses. No compounds were detected that could be specifically attributed to drum 68660 (*e.g.* in the sample extract, the compounds dodecanol, hexadecanoic acid, methyl ester, and octadecandienioc acid, methyl ester were tentatively identified by match with library spectra; however, these compounds are most likely attributed to the environmental background and not to the contents of drum 68660). Figure 1 shows the total ion chromatogram produced from GC/MS analysis of Sample FSC 14-14-1-A-1.

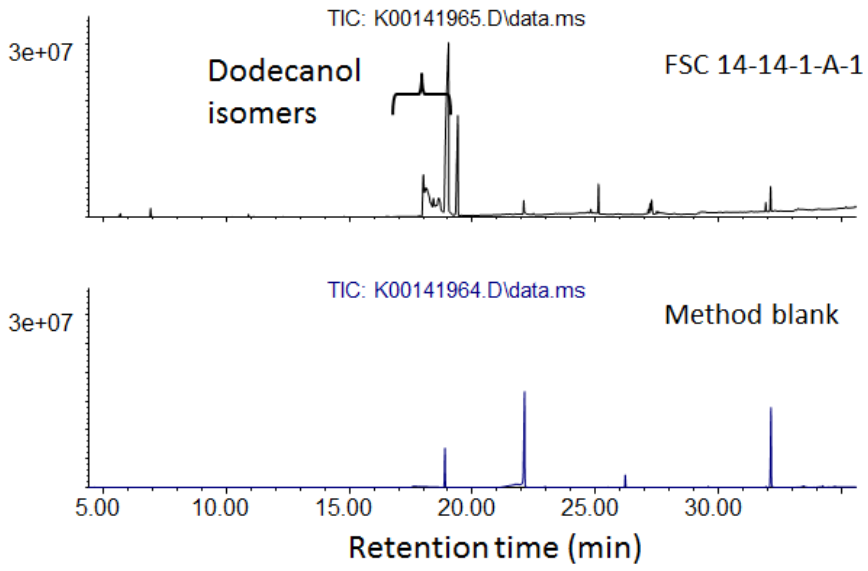


Figure 1. Total ion chromatograms from GC/MS analysis of Sample FSC 14-14-1-A-1 (top) and method blank (bottom).

The samples were also analyzed by LC/MS, using a high-resolution mass spectrometer capable of tandem mass spectrometry experiments, in both positive and negative electrospray ionization modes. Unlike GC/MS, no LC/MS libraries are available to assist in the identification of unknown compounds; however, by exploiting accurate mass measurements and MS/MS fragmentation interpretation, the presence of targeted compounds could be detected. Using targeted analysis, triethanolamine was tentatively identified in the sample using positive electrospray ionization. This compound is considered to be "tentatively identified" because its molecular weight/formula and the data associated with its MS/MS spectrum are consistent with its identification as triethanolamine (measured *m/z* value for the $[M+H]^+$ ion agreed within 2.0 ppm of the expected *m/z* value); however, confirmation of its identity based on the data generated by an authentic reference material was not performed (*i.e.* retention time and mass spectral data matches between the unknown chemical and an authentic reference chemical were not made); see MS/MS mass spectrum in Figure 2.

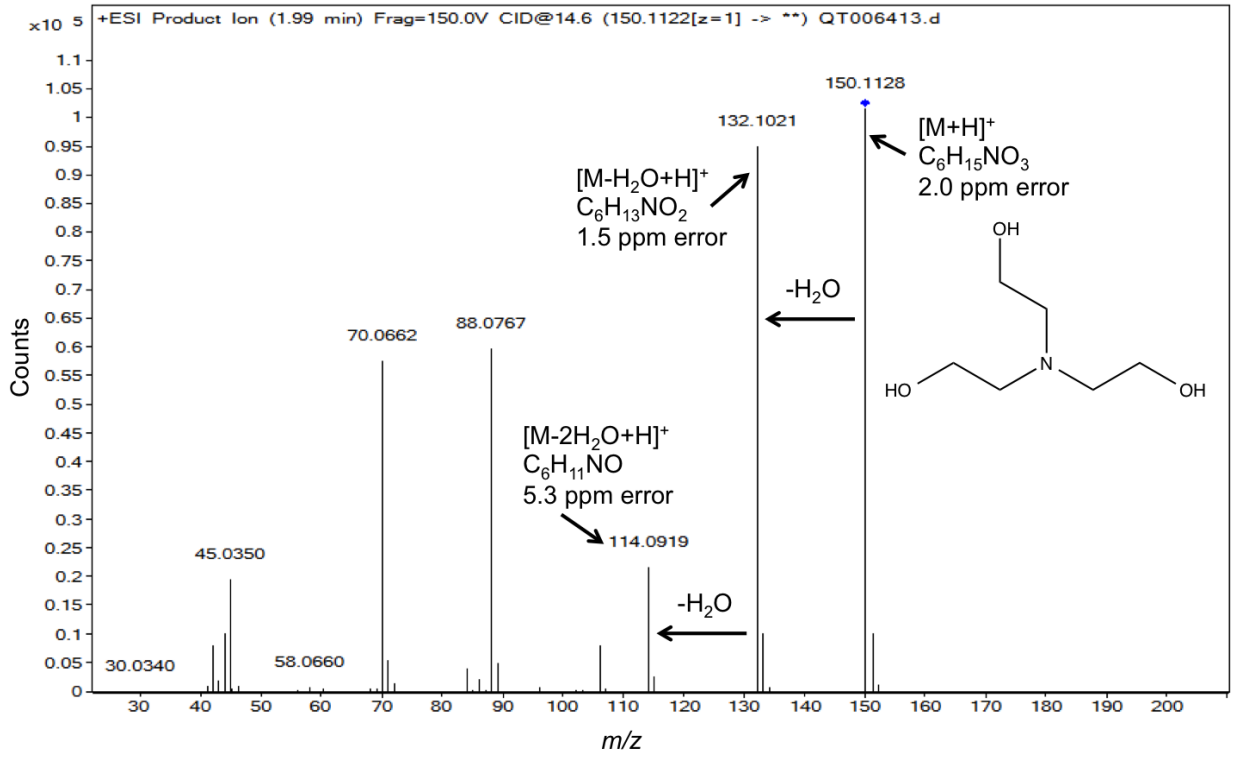


Figure 2. Product ion (LC/MS/MS) spectrum of compound tentatively identified as triethanolamine.

Using negative electrospray ionization mode, a wide peak at a retention time of 16.73 min appeared to be consistent with the tentative identification of dodecanol isomers by GC/MS; this peak yielded an $[M-H]^-$ ion, the *m/z* value of which agreed within 0.54 ppm of the expected *m/z* value for dodecanol.

Negative electrospray ionization LC/MS also appeared to show the presence of compounds that were tentatively identified as hexose (see MS/MS spectrum Figure 3) and hexose oligosaccharides with up to five degrees of polymerization. While definitive structures (isomers) of the hexose oligosaccharides could not be identified, it was determined that their $[M-H]^-$ ions had mass errors of 3.4 ppm, 0.59 ppm, 0.79 ppm, 0.90 ppm, and 2.4 ppm, for one through five degrees of polymerization, respectively.

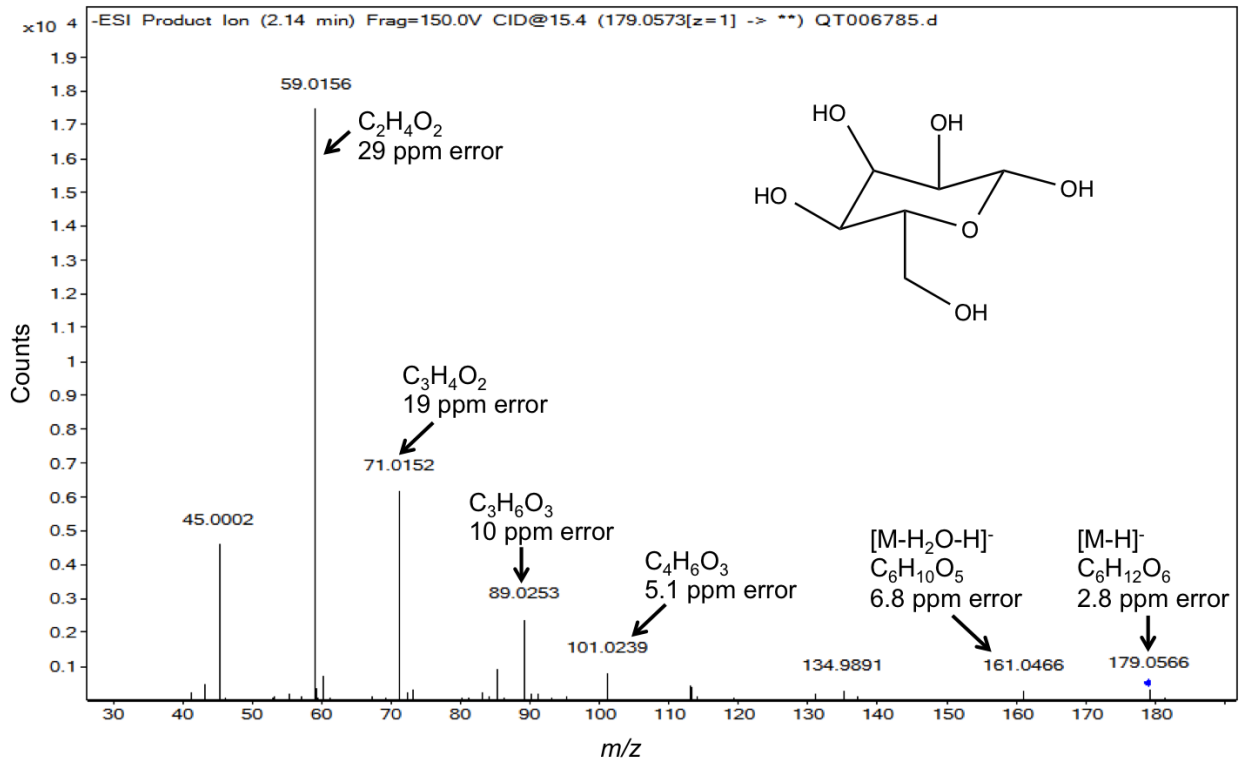
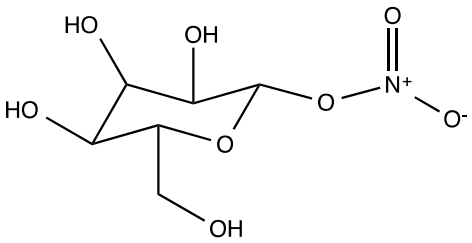


Figure 3. Product ion (LC/MS/MS) spectrum of compound tentatively identified as hexose.

Negative electrospray ionization LC/MS was also performed because it was expected to provide a better response for nitrated organic compounds. Using this mode of analysis, the data was processed using an algorithm that would allow the detection of compounds having a neutral loss of an NO_2 group (*i.e.* a targeted analysis for nitrated compounds, using a neutral loss of *m/z* 45.9929 in auto MS/MS spectra). Using this analysis strategy, no nitrated compounds were identified in the sample. Nitrated hexose, a speculated reaction product shown in Figure 4, was specifically targeted for detection and was not found in the sample. Nitrated triethanolamine was also targeted for detection and was also not found in the sample.



Chemical Formula: $C_6H_{11}NO_8$
Exact Mass: 225.0485
Molecular Weight: 225.1530

Figure 4. Structure of hypothesized, but not detected, nitrated hexose.